



Antibacterial activity of coumarine derivatives synthesized from 4-amino-7-chloro-2-oxo-2H-chromen-3-carbaldehyde and comparison with standard drug

Aziz Behrami 1
Kozeta Vaso 2

1 Faculty of Food Technology University of Mitrovica Isa Boletini

2 Faculty of Natural Science University of Tirana

ABSTRACT

In present paper, we report the organic syntheses of three compounds from 4-Amino-7-chloro-2-oxo-2H-chromen-3-carbaldehyde and describe the results of antibacterial activity of purified compounds. 7-Chloro-4-[(2-hydroxy-benzylidene)-amino]-2-oxo-2H-chromen-3-carbaldehyde (1a), 4-[(2-Hydroxy-benzylidene)-amino]-7-(4-hydroxy-phenylamino)-2-oxo-2H-chromen-3-carbaldehyde (2a), ([4-[(2-Hydroxy-benzylidene)-amino]-7-(4-hydroxyl-phenylamino)-2-oxo-chromen-3-ylmethylene]-amino)-acetic acid (3a), have been synthesized and characterized using melting points, IR spectra, ¹H-NMR and ¹³C-NMR spectra. The antibacterial activity of synthesized compounds and streptomycin and cefalexine at concentrations of 2mg/ml, 3mg/ml and 5mg/ml, have been evaluated against three strains of bacterial culture; *Staphylococcus aureus*, *E.coli* and *Bacillus cereus*. The compounds show bacteriostatic and bactericidal activity.

Keywords: Coumarine derivatives, antibacterial activity, IR, ¹H-NMR, ¹³C-NMR, Streptomycine.

INTRODUCTION

Starting from 4-Amino-7-chloro-2-oxo-2H-chromen-3-carbaldehyde (a); derivatives (1a,2a,3a) are synthesized. Coumarin derivatives are large group of heterocyclic with oxygen as heteroatom. Coumarin is a chemical compound (specifically, a benzo- α -

pyrone) found in many plants notably in high concentration in the tonka bean (*Dipteryx odorata*), vanilla grass (*Anthoxanthum odoratum*), woodruff (*Galium odoratum*), mullein (*Verbascum spp*), and sweet grass (*Hierochloa odorata*). Coumarin and their derivatives have shown various biological activities. Their fame has come mainly from their antithrombic, antiinflammatory, vasodilatory, and antiviral activities. Other several coumarin derivatives have antimicrobial properties (Z.M.Nofal; M.El-Zahar; and S.Abd El Karim), with reflux and condensation we have synthesized some new coumarin derivatives and to investigate their antibacterial activity against *Staphylococcus aureus*, *E.coli* and *Bacillus cereus*. The antibacterial activity of synthesized compounds is compared with antibacterial activity of Cefalexine and Streptomycine.

EXPERIMENTAL SECTION

Experimental Chemistry

7-Chloro-4-[(2-hydroxy-benzylidene)-amino]-2-oxo-2H-chromen-3-carbaldehyde (1a), 4-[(2-Hydroxy-benzylidene)-amino]-7-(4-hydroxy-phenylamino)-2-oxo-2H-chromen-3-carbaldehyde (2a), ([4-[(2-Hydroxy-benzylidene)-amino]-7-(4-hydroxyl-phenylamino)-2-oxo-chromen-3-ylmethylene]-amino)-acetic acid (3a) are synthesized.

Measurement

The identification of derivatives 4-hydroxy-chromen-2-one (1a,2a,3a), is made by using melting point, IR, ¹H NMR, ¹³C NMR spectra and elemental analysis. Melting point was determined on a Electrothermal apparatus (Fisher Scientific 2555) in an open capillary tube and are uncorrected. Infrared spectra were recorded in cm⁻¹ for KBr pellets on a FT-IR Shimadzu 8400S spectrophotometer with resolution 4 cm⁻¹. ¹H NMR spectra were recorded on a Bruker UNITY plus-500 'NMR 1' spectrometer using DMSO-d₆ as the solvent and TMS as the internal reference standard ($\sigma = 0.00$ ppm). Chemical shifts are expressed in δ ppm. Mass spectra were taken on a LKB 9000 mass spectrometer. Elemental analysis was performed on a Perkin-Elmer 240 BCHN analyzer. The purity of the compounds (synthesized) was routinely checked by TLC using Merck Kieselgel-60 (F-254) and benzene, toluene, glacial acetic acid (80:10:10) as mobile phase. The spots were exposed in iodine vapour for visualization.

Preparation of 7-Chloro-4-[(2-hydroxy-benzylidene)-amino]-2-oxo-2H-chromen-3-carbaldehyde (1a)

For this synthesis is used as substrate in a 100 ml flask mixed 5 g 4-amino-7-chloro-2-oxo-2H-chromen-3-carbaldehyde, 10ml Dioxane, 0.5ml Et₃N

The mixture was refluxed at 100 °C for 5h. The obtained crystals yellow are filtered and rinsed with ethanol and dried at room temperature. Recrystallization from absolute ethanol gave a yellow product of 80% yield, melting point 327 °C.

(Scheme.1)



Preparation of 4-[(2-Hydroxy-benzylidene)-amino]-7-(4-hydroxy-phenylamino)-2-oxo-2H-chromen-3-carbaldehyde (2a)

In a 100 ml flask were mixed 4g 7-Chloro-4-[(2-hydroxy-benzylidene)-amino]-2-oxo-2H-chromen-3-carbaldehyde with 10ml Ethanol, 3g amino phenol. The mixture was refluxed at 90 °C for ca. 6h.

The obtained red crystals are filtered and dried at room temperature. Recrystallization from C₂H₅OH gave red crystals product of 70 % yield, melting point, 399 °C.

(Scheme 2).

Preparation of ([4-[(2-Hydroxy-benzylidene)-amino]-7-(4-hydroxyl-phenylamino)-2-oxo-chromen-3-ylmethylene]-amino)-acetic acid (3a)

In a 100 ml flask were mixed 3g of 4-[(2-Hydroxy-benzylidene)-amino]-7-(4-hydroxy-phenylamino)-2-oxo-2H-chromen-3-carbaldehyde, with 10 ml Ethanol. The mixture was refluxed at 100 °C in water bath for ca. 8 h. The flask was placed in an ice bath for 1h until yellow crystalline precipitate was formed. After filtration the product was recrystallized from C₂H₅OH. The recrystallization gave a red product at 70% yield, melting point; 459 °C.

(Scheme 3).

Table-1 Analytical data

Compd m.p M.F Elemental analysis. Calculated : Found (calc) % C H N O Cl

1a 327 °C C₁₇H₁₀ClNO₄ 62.30 62.28 3.08 3.00 4.27 4.20 19.53 19.50 10.8 10.7

2a 399 °C C₂₃H₁₆N₂O₅ 69.00 68.5 4.03 4.00 7 6.95 19.98 19.98

3a 459 °C C₂₅H₂₁N₃O₆ 63.35 63.30 4.61 4.58 9.15 9.12 20.89 20.87

Antibacterial activity

The purified synthesized compounds (1a, 2a, 3a) was subjected to test in vitro its antibacterial activity against three bacterial cultures; Staphylococcus aureus, E. Coli and B. cereus. Antibacterial activity of compounds was investigated applying the Kirby-Bauer method or disc method (d=5.5 mm max. capacity 10 µg)

Table 2 Antibacterial activity- Staphylococcus aureus

Inhibition zone (mm)

Compound 2mg/ml 3mg /ml 5mg/ml

1a 11 15 17

2a 12 16 19

3a 13 16 20

Cephalexine 8 8 8

Streptomycin 20 20 20

Table 3 Antibacterial activity – E. Coli Inhibition zone (mm)

Compound 2mg/ml 3mg /ml 5mg/ml

1a 7 12 16

2a 8 14 18

3a 9 18 17

Cephalexine 8 8 8

Streptomycin 20 20 20

Table 4 Antibacterial activity – Bacillus cereus Inhibition zone (mm)

Compound 2mg/ml 3mg /ml 5mg/ml

1a 7 12 17

2a 8 13 18

3a 11 17 19



Cephalexine 999

Streptomycin 202020

RESULTS AND DISCUSSION

By reacting equimolar amounts of 4-Amino-7-chloro-2-oxo-2H-chromen-3-carbaldehyde and corresponding reagents (according scheme 1) under reflux reaction conditions product 1a is synthesized in 80 % yield.

By reacting equimolar amounts 7-Chloro-4-[(2-hydroxy-benzylidene)-amino]-2-oxo-2H-chromen-3-carbaldehyde and corresponding reagents (according scheme 2) under reflux reaction conditions product 2a is synthesized in 70 % yield.

By reacting equimolar amounts of 4-[(2-Hydroxy-benzylidene)-amino]-7-(4-hydroxy-phenylamino)-2-oxo-2H-chromen-3-carbaldehyde and corresponding reagents (according scheme 3) under reflux reaction conditions product 3a is synthesized in 80% yield.

The structure of 4-Amino-7-chloro-2-oxo-2H-chromen-3-carbaldehyde derivatives (1a,2a,3a) were determined from their IR, ¹H NMR, ¹³C NMR spectra and their melting points as follows.

For (1a); IR bands (KBr, cm⁻¹) 3850-2400 cm⁻¹ (OH), NH; 2910 cm⁻¹ (C-HO stretch.), 1720 cm⁻¹ (C=O), 1600 (C=C stretch.), 750 cm⁻¹ (C-H bend.) 600 cm⁻¹ (C-Cl stretch.)

¹H NMR (DMSO-d₆) δ ppm; 9.68 ppm s(H,CHO), 7.21-7.53 t(H,aromatic), 5.18 s (H,OH) 4.0

¹³C NMR (DMSO) δ ppm; 166.9 ppm (C-Cl), 162 ppm (C,COO); 152 ppm (C,C-O); 133.4 (C,C-Cl); 121.7, 125.6, 128.0 (3C-aromatic)

For (2a) IR bands (KBr, cm⁻¹) 3400 cm⁻¹ (OH) 3200 cm⁻¹ (N-H stretch.), 3000 cm⁻¹ (C-H stretch.), 3200 cm⁻¹ (N-H stretch.), 2730 cm⁻¹ (C-H stretch.), 1725 cm⁻¹ (C=O stretch.), 1600 cm⁻¹ (C=C stretch.), 1050 cm⁻¹ (C-O stretch), 750 cm⁻¹ (C-H bend.)

¹H NMR (DMSO-d₆) δ ppm 6.37, 6.39, 7.41 t(3H aromatic) 5.0 (H,OH), 4.0 d(H,NH), 5.0 ppm (H,OH), 4.0 ppm s(NH)

¹³C NMR (DMSO) δ ppm 181 ppm (C,C-NH), 178 ppm (C,CHO), 162 ppm (C,COO), 151 ppm (C,C-O), 105, 109, 116, 127 ppm (4C aromatic)

For (3a) IR bands (KBr, cm⁻¹) 3280 cm⁻¹ (O-H stretch.), 3180 cm⁻¹ (NH stretch.), 3000 cm⁻¹ (C-H stretch.), 2400 cm⁻¹ (O-H carbocyclic), 1760 cm⁻¹ (C=O stretch.), 1650 cm⁻¹ (C=N stretch), 1710 cm⁻¹ (C=O), 1020 cm⁻¹ (C-O), 750 cm⁻¹ (C-H bend.)

¹H NMR (DMSO-d₆) δ ppm 7.4, 6.5, 6.4 (3H aromatic), 5.0 (H,OH), 4.0 s(H,NH), 3.53 ppm t(CH₂), 2.65 ppm t(3H,CH₃N), 11.40-155 ppm t(4H,2CH₂)

¹³C NMR (DMSO) δ ppm 176.0 ppm (C,COOH), 167. ppm (C,C-NH), 162.0 (C,C=O), 151.7 ppm (C,C-O), 127, 109, 105 ppm (3C aromatic), 51.6 (C,C-N), 46.6 (C,C-N), 62.7 (C,C-OH), 30.6, 27.8 ppm (C,CH₂)

CONCLUSION

From the results the following conclusion were drawn: The study provides the first evidence that compounds (1a,2a,3a) obviously inhibit the growth of *S.aureus*, *E.coli* and *B.cereus*.

The compounds (1a,2a,3a) compared with the antibacterial activity of Streptomycin in *S.aureus*, *E.coli* and *B.cereus*.

This study provided the first evidence that these compounds 1a,2a,3a showed a significant antibacterial effect against *S.aureus*, *E.coli* and *B.Cereus*.

The chemical structures of synthesized compounds were determined according to extensive NMR experiments and published data.

Acknowledgements

The authors thank Prof. Branko Stanovnik, University of Ljubljana and its laboratory staff for ¹H NMR spectrum and elemental analyses.

REFERENCES

1. S.Govori; V.Kalaj; V.Rapic; L.Kalaj and S.Dakovic, Heterocycl. Commun. 2002., (8), 129
2. B.Stanovnik; H.Susachitzky and E.F.Scriven, Progress in Heterocyclic Chemistry, Pergamon Press, Oxford, 1993, (5), 75-146.
3. S.H.Lee; D.-S.Shin; J.-S.Kim; K.-B. Oh and S.S.Kan, Arch. Pharm. Res 2003., 26.
4. Vyas KB; Nimavat; KS, Jani GR; Hathi MV, 2009) Synthesis of antimicrobial activity of coumarine derivatives
5. metal complexes: An in vitro evaluation. Orbital, (1), 183-192.
6. Abyshev AZ; Gimdein Va; Semenov EV; Agaev EM, Abdulla-zade AA, Gueinov AB, 2006



7. A.Behrami;K.Vaso;I.Krasniqi, J. Int .Environ .Appl.Sci.2010, (5).247 .
8. 7.M.D.Aytemir ; R.C.Hider ; D.D.Erol ; M.Ozalp; and M.Ekizoglu .Turk.J.chem., 2003,445.
9. 8.M.M.El.Saghier;M.B.Naili;B.Kh.Rammash; N.A.Saleh and K.M.Kreddan, Arkivoc, 2007,83.
10. Z.M.Nofal ;M.El-Zahar; and S.Abd El Karim, Molecules, 2000,(5).99 .
11. 10.Chaluvaraju KC and Ishwarbhat K.Asian , J Chem 2008; (20), 4335.
12. 11.Rajan Ra Kali ;Jubie S,Grworamma B; and Suresh B,Asian J Chem 2008:(20), 5289.
13. 12.Ali Mohammed Ashraf ; and Sharayar Mohammed . Boorg Med Chem. Lett 2009;(17),3314.
14. 13.Pandeya SN;Lakshmi VS Aandey A. Indian J Pharma Sci 2003;(65):213

